Development of S_1 Families in Garlic

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Abstract

Production of true seed in garlic (*Allium sativum* L. and *A. longicuspis* L.) has been known for several years. Although release of seed derived varieties may be nearing reality, there are no reports on inheritance patterns of plant characteristics for this species. The lack of information may be due to difficulties in producing inbred families. From 1999 to 2001, over 500 garlic plants were self-pollinated to generate S_1 progenies for genetic studies. In all years, the number of S_1 seeds harvested was low. About 9% of the self-pollinated plants produced from 10 to 73 seeds each, and only one produced a total of 271 seeds. The seed germination rate averaged 8%, and the number of S_1 plants established was even lower. Phenotypic characteristics among plants of the S_1 families that were different from the parents included the absence of viable pollen, the presence of yellow anthers, and different bulb size. Many seedlings either had severe chlorophyll deficiencies or truncated roots or did not grow beyond the first leaf stage. Despite the deleterious characteristics, S_1 bulbs of a few families were successfully harvested and they represent valuable materials for studies on garlic genetics.

INTRODUCTION

Discovery of seed-fertile garlic clones provided a base for generative reproduction of this crop. Tremendous improvement in the number of true seed produced (Etoh et al., 1988; Inaba et al., 1995; Jenderek, 1998) compared to the initial amount of seeds reported (Konvicka, 1984; Pooler and Simon, 1993), created an assumption that seed derived and seed propagated garlic cultivars will be developed in the near future. Perhaps the large number of true seeds gave rise to many seed-derived families, which represented good selection material for garlic breeding. One of the main obstacles in cultivar development in garlic is the lack of information on the genetics of this species.

The objective of this study was to evaluate the possibility of producing S_1 garlic seeds from publicly available clones and developing S_1 families for future genetic studies.

MATERIALS AND METHODS

Plants used to produce S_1 seeds were selected from the garlic collection at the USDA, ARS, Western Regional Plant Introduction Station, Pullman, Washington and from the USDA, ARS collection, Madison, Wisconsin. From one bulb, a unit, 5-10 cloves were planted in the field in a semi circular arrangement, at the USDA, National Arid Land Plant Genetic Resource Unit, Parlier, California. Planting was done in October in the year preceding self-pollination. Bulbils present in inflorescences were manually removed. Before anthesis, flower stalks of each unit were isolated within an insect exclusion cage. Flowers within each unit were pollinated using blue bottle flies (*Protophormia terraenovae*), and the number of units pollinated from 1999 through 2001 varied in each year.

S_1 seeds produced by each unit were germinated in vitro under aseptic conditions. Seedlings with 2 to 3 leaves were planted into artificial medium soil mix under screenhouse conditions. At the 3 to 4 leaf stage, plants were transplanted to the field at 5 cm apart. Selected plant characteristics were evaluated under field conditions.

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RESULTS AND DISCUSSION

During the 3 years of this study, flowers on umbels of 502 garlic units were pollinated. From this number, 99.2% units produced less than 50 seeds (Table 1). The remaining 0.8% produced from 50 to 180 seeds. The three best units P61-18, P47-116 and P77-581 (selections from PI 540316 and PI 540337) produced 271 (180 seeds in the first year and 91 seeds in the second year), 73, and 71 seeds, respectively. The average seed germination rate across the S_1 seeds produced was 8.2%, and ranged from 0 to 59.4%. For S_1 families that had plants established in the field, the best were P61 and P77 (both selections from PI 540316), with 56.8 and 29.6% of plants established, respectively. However, across all families, the average rate of S_1 plants established in the field was 6.1%. For P47 only 11.0% of the 73 S_1 seeds harvested produced viable plants in field (Table 2).

Several S_1 plants established in the field expressed different characteristics than the original parental clones. All parents in this study bolted, produced umbels with flowers and had purple anthers and petals. They were all male fertile. In the progenies, in almost 70% of the families established in field, bolting and non-bolting plants were observed. Many S_1 plants had purple petals as in the parents, but lilac and white petals were also occasionally observed. Plants with purple petals exhibited various shades of the color, depending on the color of the petal tips and veins. Some progenies originating from parents with purple pedicels had green pedicels. In the group of plants with the purple pedicels, in the 2 largest families (P61 and P77), various intensities of the color from slight lilac to dark purple were observed.

In both S_1 families, progenies with purple and yellow anthers were present, yet the parent plants produced only purple anthers. Plants were evaluated as sterile when they 1) had shrunken, deteriorated anthers without visible pollen, 2) had non-stainable, crescent shaped grains, or 3) were without any pollen grains on microscopic slides. Within both populations, male fertile and male sterile plants were present. For some plants, the characteristic was not described due to flower deformations or a lack of flower stalk development. The majority of plants evaluated in P77 were male sterile, but in the P61 most of the plants were male fertile. All plants in the P77 family developed more leaves/plant, and the leaves were longer than the parents. Although the majority of the S_1 plants exceeded the length of the parents, a group of much shorter plants than the parent was observed. Numerous plants with additional leaves in the primary leaf axils were observed in the P61 family, and to a lesser extent in P77. For commercial production, plants with additional leaves are considered as defects because bulbs of such plants form additional, small cloves, which often are the cause of irregular bulb shape and are not desirable for fresh market sale. In both S_1 families evaluated, only one bulb per family exceeded the size of the parent bulbs. The majority of the S_1 bulbs were smaller than in the parents or of the same size.

Observation of the S_1 seed germination in vitro revealed the presence of seedlings that were not able to release their radicle from the seed coat. Such seedlings usually did not successfully develop. Some seedlings emerged with a truncated radicle, and the first leaf did not elongate beyond 2 cm. Lack of chlorophyll development (various degrees of pale yellow to lime green color) of the first 2 leaves was observed in up to 50% of some S_1 families. The most chlorophyll deficient seedlings died at the in vitro stage, whereas the darker ones were established in the screenhouse. Some plants with lime green color leaves developed a small bulb in the field. Several seedlings did not grow beyond 4 cm long and their leaves senesced without forming a bulb. Some seedlings developed 2 to 6 mm wide, undivided bulbs, but rarely would the small bulbs develop into a plant. This was observed both in plants grown in test tubes and in the screenhouse. Some well established seedlings in the screenhouse had short leaves ($\leq$ 10 cm), which did not elongate further in the field, and later formed small, undivided bulbs. Such plants did not develop a flower stalk. A few plants resembled the growth habit of cultivated leek. They did not develop a bulb, and after two vegetative seasons the majority of such plants died. Flower stalks of the S_1 plants exhibited various degrees of speed in senescence. In some plants senescence started in the stalk looping stage, and progressed rapidly to a light brown color before flowers developed. Anomalies in flower umbels included absence of flower buds, presence of only a few buds (which usually did not develop to full flowers), growth of a second tier of flower buds that developed in the ovary of the first flower tier, and the development of bulbils in flowers. Those characteristics were observed in open-pollinated progenies as well, but at a much lower level.

Literature on characteristics observed in seed derived garlic plants could not be found. Katharzin and Katharzin (1978) obtained a few seeds from a single garlic plant in the field, and perhaps this event could be considered as the first published self-pollination effort in garlic. However, it is not known if this plant was the only fertile plant grown in the field at this time, and there was no description of the progeny characteristics. Hong and Etoh (1996) obtained 346 seeds from 17 self-pollinated clones, and the number of seeds ranged from 0 to 103 per clone. Out of the 346 seeds, three seedlings developed to a stage of 2-3 leaves and than died. In the two largest S_1 families in this study, the number of plants developed and evaluated was low, and a large group of plants was not evaluated for important breeding characteristics such as male fertility and bulb size, because of absence of flower stalks, flower defects or not incompleted vegetative stage. Knowledge of the inheritance pattern is very important for breeding and seed production purposes, especially in development of male fertile and male sterile lines. A relatively large number of plants did not bolt at all during two vegetative seasons. It will be interesting and important for future garlic variety development to study the inheritance of non-bolting garlic plants, and to determine whether they can be derived from true seeds.

CONCLUSIONS

In this study, several S_1 garlic seeds and plants were produced. The S_1 seed germination rate and the number of S_1 plants established in the field were low, but two S_1 families were established in field. Several S_1 progenies had morphological defects, which contributed to the low number of plants established in field. Some of the S_1 progenies exhibited characteristics different from the parents used to develop the families. Those included different petal and anther color, presence of non-bolting and male sterile plants, presence of plants with different leaf number, leaf length, and bulb size. Development of S_1 families, and ultimately new genetic clones, is challenging but possible. Selected plants derived from sexual propagation will be used in studies on garlic genetics to support variety development and improvement of seed production of this species.

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Tables

Table 1. Number of S_1 seeds produced by different garlic accessions and units in 1999-2001.

Year	Number of different accessions	Number of units pollinated	Number of units with S_1 seeds			
			0-30	>30-50	>50-100	>100
1999	2	9	7	1	0	1
2000	17	409	400	6	3	0
2001	19	84	83	1	0	0
Total number of units		502	490	8	3	1
Percent (%) of units			97.6	1.6	0.6	0.2

Table 2. Seed germination and number of S_1 plants established in field for selected garlic families.

Accession	Country of origin	S_1 family	Number of S_1 seeds	Germ-ination (%)	S_1 plants established in field	
					Total	% of total S_1 seeds[1]
PI 540314	USSR	P36-39	36	25.0	4	11.1
PI 493099	USSR, Moldavia	P41-535	11	45.4	2	18.1
PI 540335	Czechoslovakia	P46-94	11	36.4	2	18.2
PI 540337	Czechoslovakia	P47-114	23	43.5	5	21.7
PI 540337	Czechoslovakia	P47-116	73	16.4	8	11.0
PI 540337	Czechoslovakia	P47-132	40	30.0	4	10.0
PI 540337	Czechoslovakia	P47-103	21	38.1	4	19.0
PI 540356	USSR, Georgia	P51-209	23	30.4	1	4.3
PI 540356	USSR, Georgia	P51-225	39	20.5	4	10.3
PI 540356	USSR, Georgia	P51-241	20	30.0	2	10.0
PI 540356	USSR, Georgia	P51-245	18	50.0	5	27.8
PI 540356	USSR, Georgia	P51-288	18	38.9	4	22.2
PI 540357	USSR	P52-274	23	30.4	1	4.3
PI 540357	USSR	P52-325	36	19.4	5	13.9
PI 540357	USSR	P52-335	42	16.7	3	7.1
PI 540316	USSR	P61-18	271	59.4	154	56.8
PI 540316	USSR	P77-581	71	35.2	21	29.6
PI 540316	USSR	P77-588	27	25.9	2	7.4
DDR 6038	USSR, Georgia	P79-459	19	26.3	1	5.3
DDR 6038	USSR, Georgia	P79-473	31	16.1	1	3.2
DDR 6038	USSR, Georgia	P79-484	22	36.4	5	22.7
DDR 6038	USSR, Georgia	P79-491	40	22.5	6	15.0
DDR 6038	USSR, Georgia	P79-494	52	13.5	5	9.6
DDR 6038	USSR, Georgia	P79-503	28	28.6	6	21.4
PI 383819	Yugoslavia	P82-422	31	6.4	1	3.2

[1] The percentage of total S_1 seeds that resulted in plants established in the field.